ANALYSIS OF POPULATION DEVELOPMENT IN DAPHNIA AT DIFFERENT TEMPERATURES

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INTRODUCTION

The purpose of this study was to analyze the development of Daphnia populations under controlled conditions in which temperature was the chief variant. It was proposed to investigate not only the effect of temperature upon the rate of increase, but also its influence upon subsequent changes in the numerical strength of the population. The original intention was to measure the effect of temperature by comparing the equilibrium values, i.e. asymptotes, attained by populations at different temperatures, and through further experiments to identify the processes by which temperature might act to bring about the observed differences. However, the type of population growth curve obtained precludes the comparison of asymptotes and necessitates a brief historical sketch of population studies in general and the curves developed from them, in addition to a review of previous research upon the effects of temperature.

Since the animal chosen for the investigation is a planktonic form, the analysis of the relation of temperature to the development of a population acquires additional interest from an old controversy. It has long been held that the polar regions support a more abundant, if a less diversified, fauna and flora than do the tropics. This contention has been stressed especially in connection with the latitudinal distribution of plankton, and a number of theories have been advanced relating temperature, directly or indirectly, to the density of planktonic populations. The relation of the present study to this problem and other possible applications will be dealt with in the discussion.

HISTORICAL BACKGROUND

The logistic equation and its sigmoid curve, rediscovered by Pearl and Reed (1920), have been applied to the study of human populations (Pearl, 1925) and experimental populations of a variety of organisms, including yeast cells (Clark, 1922; Richards, 1928), diatoms (Ketchum and Redfield, 1938), infusorians (Robertson, 1921, 1923), and flour beetles (Chapman, 1928; Holdaway, 1932). Indeed no population study under controlled environmental conditions has demonstrated any other type of population growth curve. It should be noted, however, that the interest of investigators of experimental population development has been focussed almost exclusively on the early parts of the growth curve, with very little regard for the important part of the history which follows the initial period of increase. Although the definition of a logistic curve requires an upper asymptote, some workers have followed the development of their experimental populations only to the point where they first approach an apparent maximum size, and in

presenting their results have termed their curves logistic. While it is true that in several studies (e.g. Pearl with Drosophila, 1925; Gause with yeast, 1932; Chapman with Tribolium, 1928) the population has maintained an upper asymptote for a period long compared to the "growth" period, it does not follow that populations of other species or under different circumstances would yield similar results. The tacit assumption that an asymptote can be calculated from the maximum size reached by a population, without experimental evidence that a state of relative equilibrium has been attained, is entirely gratuitous. It may well be that the failure to demonstrate, hitherto, a type of population growth curve that is not logistic after the initial period of increase, has been due in part to this fallacious assumption.

The rather extensive literature on population studies yields but a meager amount of information concerning the effects of temperature. The yeast *Saccharomyces cerevisiae* has been the subject of two investigations involving temperature. Richards (1928a) found that the rate of multiplication increases with temperature between 4° and 30° C.; above this range it decreases. In a more thorough analysis of the effects of temperature, Gause (1932) followed the development of yeast populations to their asymptotes and discovered that in a temperature range of 5.7° to 41.0° C. the relation between the size of the asymptotic population and the temperature can be expressed by a bell-shaped curve with the mode at about 24° C. In the same paper Gause reported that populations of Drosophila held at 29° C. attain an asymptote of 310, whereas at 30° the asymptote is only 146.

Terao and Tanaka (1928, 1928a, 1928b, 1930) attempted the study of the influence of temperature on population development in *Moina macrocopa*, but followed their population growth curves only to apparent maxima, and based their conclusions on the calculated values of undemonstrated asymptotes.

Materials and Methods

Daphnia magna would appear to be ideal material for population studies because of its size, high reproductive capacity, and parthenogenesis, which makes it easy to obtain genetically identical material. Daphnia is less easily provoked to the production of males and sexual females than other cladoceran genera (e.g. Moina), so that in a crowded population a very high percentage of the individuals are potential producers, and there is no problem of a proper balance of sexes. In these experiments the sex ratio was noted at irregular intervals, and at no time did the males constitute more than 10 per cent of the population. Since cladocerans pass their early stages in the maternal brood pouch whence they are released in active state, there are no stages (such as eggs) so small as to require special precautions against loss during transfer of the population to fresh medium.

The populations developed in 50 cc. of filtered pond water from the Middlesex Fells, in open, widemouthed glass bottles whose water-surface area was 10.9 cm.² The seeding of each bottle was two animals (parthenogenetic females) that had been released from the brood pouch within 24 hours. Each population was counted every two days, at which time the dead were removed, their number noted, and the water changed. This was done by pouring the contents of each bottle into a fingerbowl, whence the animals were transferred with a pipette to a

second fingerbowl containing about 50 cc. of fresh pond water at the same temperature, and thence into a clean bottle which was finally filled up to the 50 cc. mark with fresh pond water.   By this rinsing process the small amount of used water carried over in the pipette was greatly diluted.   Thus "conditioning" of the medium by the metabolic activities of the animals was never allowed to proceed for more than two days.

The only food used was *Chlorella pyrenoidosa*, a unicellular green alga that will grow in a thick suspension when properly cultured (in Detmer's Solution, exposed to neon light, with carbon dioxide bubbling through the medium).   It was found necessary to culture the Chlorella under sterile conditions to prevent the development of a concentrated bacterial flora in the culture flasks.   In previous experiments contamination of the Daphnia medium from this source had occasionally been sufficiently severe to injure the animals.   The quantity of food given each population was not measured by any absolute standard.   In each case it was roughly calculated, by previous experience alone, to exceed the requirements of the particular population.   This method proved entirely satisfactory, for the medium always had a distinct greenish tinge.   It was also demonstrated by simple experiments that when the concentration of the Chlorella was half as great or several times as great as the concentration that normally would have been used under the given conditions, the longevity and reproductive rate of the animals were not appreciably affected.   Therefore neither a lack nor an excess of food was ever a limiting factor in the growth of the populations.

The temperatures chosen for comparison were 12°, 18° and 25° C., covering a considerable portion of the range (8° to 28° C.) demonstrated suitable for the life and reproduction of *Daphnia magna* (MacArthur and Baillie, 1929).   At 12°, however, populations persisted for only a few weeks of faltering growth and rapidly dwindled to extinction.   Under the ecological conditions that obtained, apparently the metabolic rate was not high enough to insure the reproductive and survival rates requisite for population growth and maintenance.   In consequence the lowest temperature was abandoned and the work was limited to two temperatures, 18° and 25° C.   The populations were maintained at these temperatures (plus or minus 1° C.) by keeping them in incubators in a cold-room.

The culture bottles were placed in daylight from a north window.   However, all the populations received approximately the same amount of light.   Aside from this, no attempt was made to control light conditions, which varied from day to day and from season to season.

The only environmental agencies that suggest themselves as possible limiting factors in the growth of populations of such an animal as Daphnia are: 1. exhaustion of the food supply and 2. conditioning of the medium by the accumulation of metabolites and/or depletion of the dissolved oxygen.   Since the former was never operative in these experiments, any limitation in the increase in numbers must have been the expression of some form of conditioning of the medium, although that process was never continuously sustained for more than two days.   In an attempt to ascertain the nature of this conditioning, the concentrations of hydrogen ion, dissolved oxygen and free carbon dioxide in the culture medium were determined at various densities of population.

The pH, as determined with a Hellige Comparator, never left the range 6.9–7.1, and even within these narrow limits it was not correlated with the popula-

tion density nor with the length of time that animals had been living in the water. It can be said with a fair degree of certainty that the hydrogen ion concentration never exerted an important influence upon population growth.

The concentration of free carbon dioxide was determined by a titration method reported in a publication of the American Public Health Association (1936). Thus determined, the amount in unused pond water at 18° or at 25° was imperceptible. At the end of a two-day period, crowded populations at 18° had raised the concentration to an average of 4.97 p.p.m.; at 25°, to 5.66 p.p.m.

Winkler Method determinations of the dissolved oxygen concentrations of fresh pond water and water conditioned by large populations for two days yielded the following results: 1. At 18°, unused water 8.38 p.p.m.; after two days' conditioning 6.59 p.p.m. and 2. at 25°, unused water 7.57 p.p.m.; after two days conditioning 4.81 p.p.m. Each of these figures is the average of ten determinations.

There is no evidence available at the present time as to whether or not these slight changes in carbon dioxide and oxygen are sufficient to account for the limitation in population growth. It is also possible that the limiting factor be some metabolite such as that postulated by Brown and Banta (1932) for male production.

## Course and Analysis of Population Development

The problem of determining the influence of temperature upon population development resolves itself into two phases: 1. a descriptive study of the observable effects of temperature upon the form and dimensions of the population curve, and 2. an analysis of the processes through which the difference in temperature brings about the observed results. The present section is confined to the presentation of the factual data on the history of populations at 18° and 25° and the discussion of these growth curves. The analysis of the influence of temperature will be dealt with in later sections.

A. Observations at 25° and at 18°.

At 25°, four series of populations were started on different dates in January and February 1942. The histories of these 21 populations were recorded either until their natural extinction or until September 13, 1942, when all remaining populations were discontinued. Graph I presents the observations on a typical series, and reveals that the 25° population curve is characterized by violent and fairly regular oscillation. Instead of terminating in an upper asymptote, the first period of increase results in a pronounced peak, after which the curve drops almost to the baseline, then repeats the cycle. Typically there is no asymptote.

The majority of the populations became extinct before the experiment was terminated. Those that survived until the 234th day, when observations ceased, described, commonly, four major oscillations in numbers. The maximal size attained was a population of 126 animals.

At 18°, three series of populations, started on different dates in late March 1942, were followed until September 13, 1942, when the experiment was terminated. None of these 16 populations became extinct in the 174 days of observation. Graph II, presenting the histories of a typical series, shows that each curve described a prominent peak, followed by a gradual decrease and virtual stabiliza-
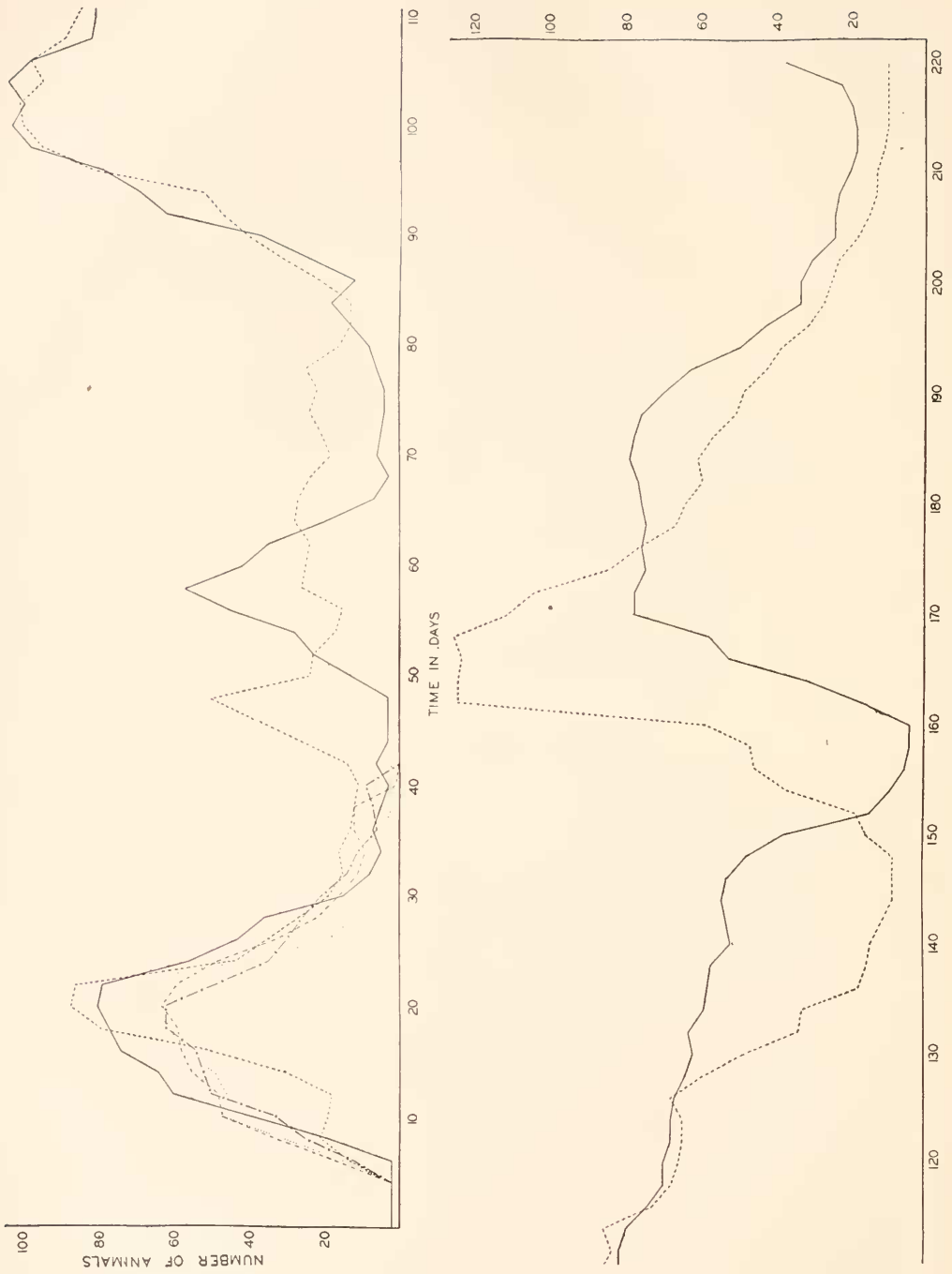
GRAPH I. Population development at 25° C. (Series C.)
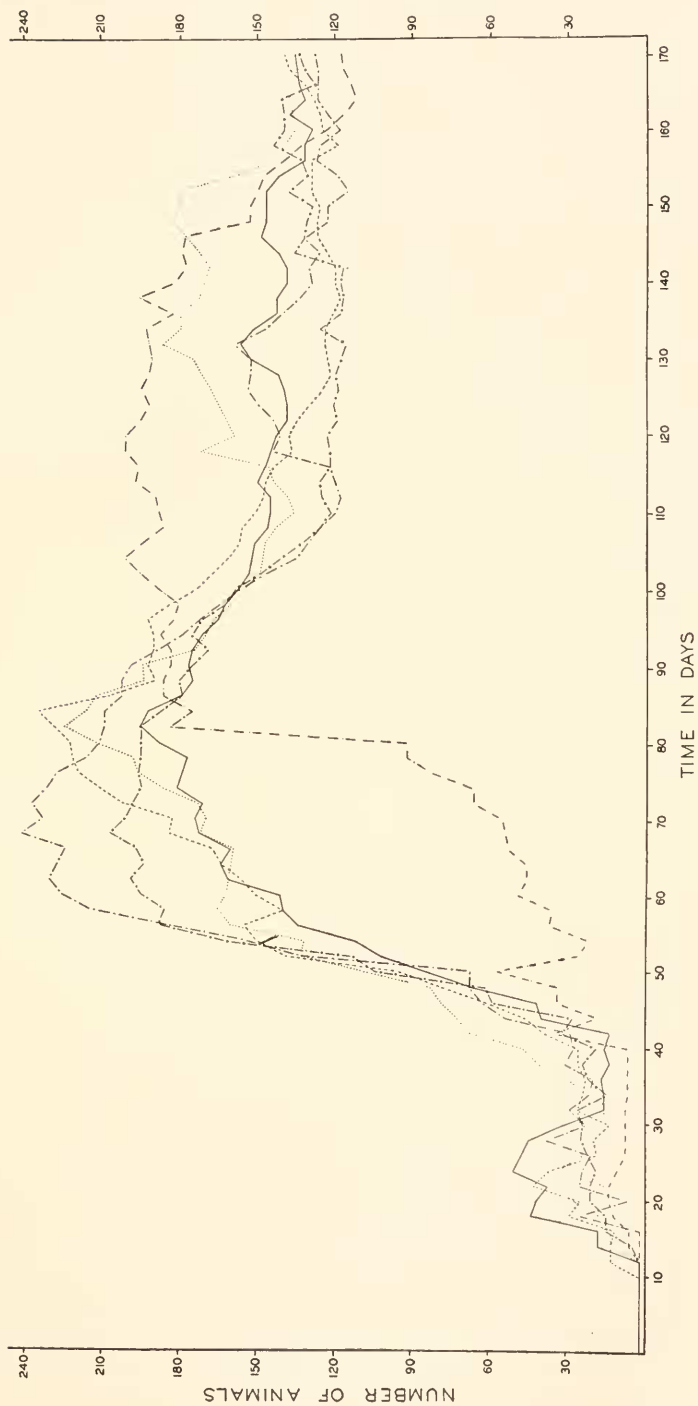
GRAPH II.   Population development at 18° C.   (Series C.)

tion or continued oscillations of relatively minor amplitude. The greatest maximum achieved was 241 animals in the 50 cc. environment, and after the major increase, a density of more than 100 animals was permanently maintained in all the populations. While the course of development at 18° is oscillatory, it differs from that at 25° in various points that will be examined later.

Preliminary experiments in which the volume of the medium used had been 100 cc. rather than 50 cc. yielded similar results with regard to the relative shapes of the curves, and population maxima of 381 individuals at 18° C. and 296 at 23° to 24° C.

B. Analysis of oscillation.

Since oscillation is especially pronounced at 25°, the discussion of this phenomenon will be illustrated with the data from that temperature. The analysis of fluctuations in the size of a population is essentially the study of changes in the ratio of births to deaths. Whereas the fundamental feature of an asymptotic population is that at some point birth rate and death rate become equal and thereafter remain constant, in an oscillatory population the curves describing birth rate and death rate repeatedly cross each other and never remain equal. The present fluctuations might be due to oscillation of the birth rate about a constant death rate, or to the converse, or to differential changes in both rates. In order to establish the cause of the fluctuations, it is therefore necessary to ascertain by which of these three methods the ratio of births to deaths varies.

Daily tabulation of births and deaths revealed that the oscillations observed in the 25° populations resulted from changes in both the number of births and the number of deaths. Periods of increase in population size were marked by a combination of high reproductive activity and low mortality; decreases were caused by increased mortality coupled with negligible reproduction. This mechanism of oscillation, in terms of the changing births/deaths ratio, is illustrated in Graph III which depicts part of the history of a representative 25° population (No. 2 in Graph I), with curves showing the numbers of births and deaths for each day of population census.

The history of one complete cycle will illustrate the reasons for these changes in the ratio of births to deaths. At the outset of an upward swing, the population consists in a few adults. Having lived the greater part, if not the whole, of their lives under favorable environmental conditions as regards crowding, these individuals exhibit a high reproductive rate. The growing population is composed of a few (two to 10 or 15) rapidly reproducing adults and their much smaller offspring. Graph III shows that the increasing population density begins to exert its harmful effects upon the reproductive rate before it affects the death rate, as it does in growing populations of Drosophila (Pearl, 1927). Thus the reproductive activity of the few adults in the population gradually dwindles, and the population reaches the maximum. The crucial and distinctive crossing of the birth and death curves at about this point is ascribed to two factors: 1. the extent of biological conditioning that occurs in the 48 hours between changes of the medium is presumably greater at this density than ever before, and 2. the cumulative adverse effects of crowding upon animals that have lived the greater part of their lives at high population densities begin to manifest themselves. The effect of these factors is sudden and severe: the death rate soars and reproduction is greatly
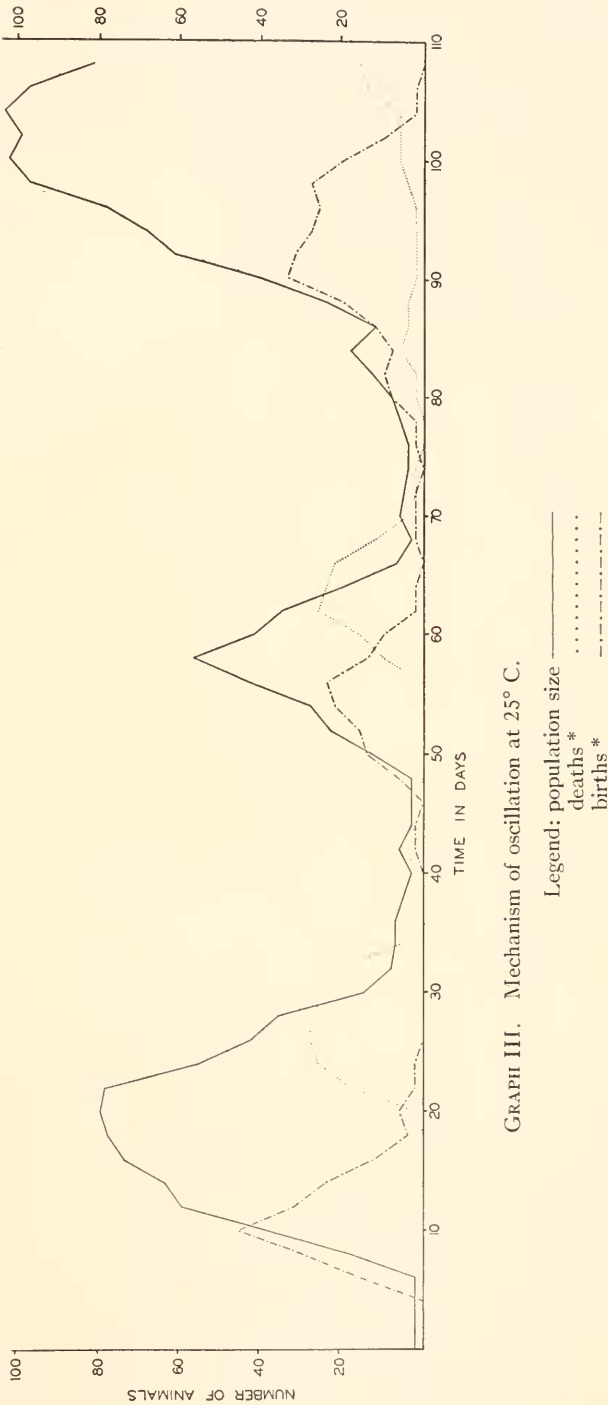
GRAPH III.   Mechanism of oscillation at 25° C.

Legend: population size ———
       deaths * · · · · · · · · · · · · ·
       births * —·—·—·—·—·—·—

* If the actual number of deaths and births occurring on each day is plotted, the resulting curves are too irregular and too low to read with ease. Accordingly, each number was doubled, and the curves smoothed by plotting the points as 3-point moving averages.

reduced. The rapidly growing population has "overshot" the density which it might theoretically be just capable of sustaining.

That the population overshoots the density of potential equilibrium does not explain why it drops dangerously close to extinction before recovering itself. At a point, for example, halfway in its descent it might be expected to rally its forces and start up again, since this same intermediate density on the upswing had been correlated with a high reproductive rate and low mortality.

In seeking the reason for the difference in performance of the two populations of equal density, it should be borne in mind that the composition of the waning population at any given point differs significantly from that of the waxing population of the same numerical strength. For example, the average size of the individuals, and hence the total biomass, of the waning population is much the greater. This fact suggests the possibility that the total metabolism of the declining population is higher than that of the growing population. If this is true, the conditioning of the medium by the accumulation of metabolic wastes or depletion of the dissolved oxygen would proceed at a greater rate in the water of the old population than in that of the younger one, and the given numerical density would exert a more severe effect upon the former than upon the latter. This might account for the difference in the subsequent histories of the two populations.

The question of comparative total metabolism was tested by determining the relative rates of depletion of dissolved oxygen in the medium. Fifty adult Daphnia chosen at random from a large culture were placed in each of six bottles with 50 cc. of pond water containing Chlorella. A similar series was made up using smaller animals from the same culture, 50 to the bottle. At the end of two days the dissolved oxygen content of the water was determined by the Winkler method, three bottles being required for each determination. The animals were then transferred to fresh pond water, 50 cc. to the bottle, for a second two-day period, after which the oxygen concentrations were again determined. The average depletion of dissolved oxygen per two-day period of conditioning was 1.19 p.p.m. in the water occupied by the 50 adults, 2.16 p.p.m. in the medium of the 50 young. In so far as the rate of oxygen depletion is a measure of metabolism, the difference between these two figures indicates that the waning population of larger biomass has a lesser, rather than a greater, total metabolism than the waxing population of equal numerical size. Thus the continued decrease of the old population cannot be assigned to a higher rate of conditioning of the medium.

There are two explanations for the continued decrease, the first of which is to be found in changes in the age structure of the population. Since the individuals of the declining population are of a greater average age than those of the increasing population, their life expectancy is of course less. Thus the difference in constitution of the waning population provides a reason for the higher daily number of deaths in this phase of the cycle.

The second reason for the continued decrease in size of the population at densities that formerly permitted increase is disclosed in the study of its previous history as compared with that of the waxing population. The components of the young, growing population had lived all of their lives, up to any density selected for comparison, at population pressures lower than the given density. They had never suffered severe crowding. But the individuals in the waning population of

identical numerical strength have lived perhaps their entire lives at densities greater than the present density.

A special experiment demonstrated that the life-long crowding experienced by the latter individuals exerts a permanent adverse effect upon their reproductive capacities. Animals were raised in a crowded condition until their first clutches were laid in the brood chamber. They were then segregated, one animal to a bottle, and their subsequent reproductive rates were compared with those of animals reared in isolation. Unfortunately this early experiment was conducted under conditions slightly different from those obtaining in the present population studies: the volume of water used was 100 cc. rather than 50; and the temperature, not controlled, varied between 22° and 27° C. The "crowded" state was a population density of 25 animals/100 cc. Animals that lived under these conditions as young (i.e. until the sixth day) and then were segregated, each into 100 cc., exhibited a reproductive rate only 62.7 per cent of that of animals that had never suffered crowding.

It should be noted that this drastic effect was brought about by crowding of only a very moderate intensity, as compared with that experienced by the declining populations under discussion. Individuals whose previous history of crowding has included population pressures ranging from 60 to 120 animals/50 cc. no doubt suffer a far more severe inhibition of reproduction. Furthermore, it is believed that subjection to high densities during early life has a lasting deleterious effect upon survival as well as reproduction, which would help to explain the persistence of a high death rate as well as a low birth rate in the shrinking population.

The effects of previous crowding may be sufficiently severe and persistent to inhibit reproduction in the waning population completely and permanently. In this event the population becomes extinct. As a rule, however, a few young are produced toward the close of the cycle, pass their juvenile stages at minimal population pressures, and attain maturity with their reproductive capacity unimpaired by crowding. From these animals stems the next growth cycle of the population.

Oscillation consists essentially in the successive "overshooting" and "undershooting" of a theoretical equilibrium density. These phenomena appear to be due to a delay, rather than a prolongation, in the manifestation of density effects. The growing population withstands a high degree of crowding with a negligible mortality. There is a lag before the effect of these population pressures is fully felt upon the death rate, which, once raised, remains high for several days while the decreasing population passes through formerly favorable densities. Likewise the reproductive rate of the increasing population is at first unaffected by high densities, but when finally checked, does not recover from the effects of crowding until long after that state of crowding has ceased to exist. Thus overshooting is occasioned by a delay in the expression of the adverse effects of high densities upon reproduction and mortality, and undershooting results from a similar lag in the manifestation of the beneficial effects of favorable densities.

C. Comparison of Oscillation at 25° and 18°.

The principal point of contrast in form of population curve at the two temperatures is the continued oscillation at 25° as compared with the tendency of the 18° curves to approach an equilibrium value.

Table I presents the duration, size-range, and mean size of the equilibria established at 18° and at 25°, and the days of the respective population histories that bounded these equilibria. After a single peak ranging from 184 to 241, oscillation at 18° was greatly reduced in all cases, six of the populations achieving nearly constant values (averaging 135.4) that they maintained until observations ceased, whereas at the higher temperature equilibria were established on only three occasions, and the general course of development was marked by a steady increase, rather than a diminution, in both the amplitude and the period of oscillation.

This progressive increase in the magnitude of oscillation at 25° is correlated with a noteworthy decrease in the rate of population extinction. Of the 21 original populations, only seven survived until the third oscillation, but of these, six were still flourishing when the experiment was discontinued. The reason for

TABLE I

Population equilibria at 18° and at 25° C.

Tempera-ture	Population	Graph	Days bounding equilibrium	Duration	Size range	Mean size
25°	Series A, No. 3	*	30–102	72 days	8– 30	21.9
	Series A, No. 3	*	132–234	102 days	30– 54	43.4
	Series C, No. 5	1	50– 80	30 days	15– 28	22.5
18°	Series A, No. 1	*	110–174	64 days	124–145	133.6
	Series A, No. 2	*	96–174	78 days	121–157	140.7
	Series B, No. 5	*	112–172	60 days	127–156	140.4
	Series C, No. 1	II	104–170	66 days	116–144	127.6
	Series C, No. 2	II	100–170	70 days	129–157	143.3
	Series C, No. 5	II	122–170	48 days	118–140	127.0

average of mean values for equilibria at 25° C. = 29.3
average of mean values for equilibria at 18° C. = 135.4

* Graph not presented in this paper.

the improved adjustment or heightened resistance to the environment apparent in the latter half to two-thirds of population history at 25° is not clear.

Barring mutations, one cannot postulate genetic improvement through natural selection, for all the animals were genotypically identical. Since the controlled ecological conditions did not vary throughout the course of the experiment, one is led to suspect some environmental factor that was not controlled. Of these, there is only one which could conceivably have evoked the observed effect. As previously stated, no attempt was made to control conditions of light. The daylight, received from a north window, varied from season to season. It is not improbable that the amount of dissolved oxygen in the medium was an important factor in population growth. The period in which the populations appear to have been better adjusted or more resistant to their environments, beginning at the end of April, coincided with the season in which a longer daily duration of effective light enabled the food-alga Chlorella to produce a greater amount of oxygen. This added daily increment of oxygen may have been sufficient to account for the

greater success of the 25° populations in the late spring and summer months. These experiments are to be repeated, at least in part, under more rigidly controlled light conditions.

If this is the correct interpretation of the increase in amplitude and period of oscillation at 25°, evidently the 18° populations were started too late in the season to experience any such improvement in environmental conditions.

A second point of comparison is found in the nearly complete and simultaneous population "overturns" at 25°, and the more continuous overlapping of generations at 18°. At the higher temperature the first peak in numbers was due entirely to the reproduction of the seed animals, which produced several broods. Typically the first generation animals died during the first population decrease, before the appearance of the third generation. The latter individuals were not produced in numbers sufficient to prevent further decrease. None of the first or second generation animals remained at the inception of the second major increase, which was brought about by the production of the fourth generation. Thus there was a minimum of overlapping of generations. Similar population overturns, more or less complete depending upon the depth and duration of the depressions, occurred between all the subsequent peaks.

In contrast, the course of development at 18° after the major upswing was not thus punctuated by the simultaneous mass replacement of one generation by the succeeding generation. A significant feature of population history at the lower temperature was the accumulation of successive generations. The simultaneous presence of animals of all ages insured a steady replacement of adults and resulted in a sustained continuity in growth and maintenance never observed at 25°.

The lack of a sufficient number of steadily reproducing adults in an 18° population occasions the spasmodic type of population growth witnessed in the first 30 or 40 days of development. This was the period during which the second generation was being produced. The relatively infrequent production of young by the two seed animals and a comparatively high infant mortality result in a highly irregular curve. The attaining of maturity by animals of the second generation caused the tremendous increase in population size which began on about the 40th day. From this point on, the overlapping of successive generations and the constant replacement of producers gives the curve its characteristic unbroken continuity.

It should be recalled that oscillation at 25° results from an alternation of fluctuations, approximately equal in amplitude, in the number of births and the number of deaths per day. The mechanism of oscillation at 18°, in terms of the births/deaths ratio, can be analyzed in Graph IV, which is similar in purpose and in method of construction to Graph III. The data are those of population No. 1 in Graph II.

Examination of these curves reveals that there was far greater variation in the number of births per day than in the number of deaths. Moreover, the two major changes in the size of the population, viz. the tenfold increase between the 40th and 56th days, and the later more gradual decrease, were correlated, respectively, with the periods of maximal and minimal numbers of daily births. While it must be conceded that the number of deaths per day was slightly greater while the population decreased than during the period of increase, both of these levels on the deaths curve are equalled in other parts of that curve, and the difference be-
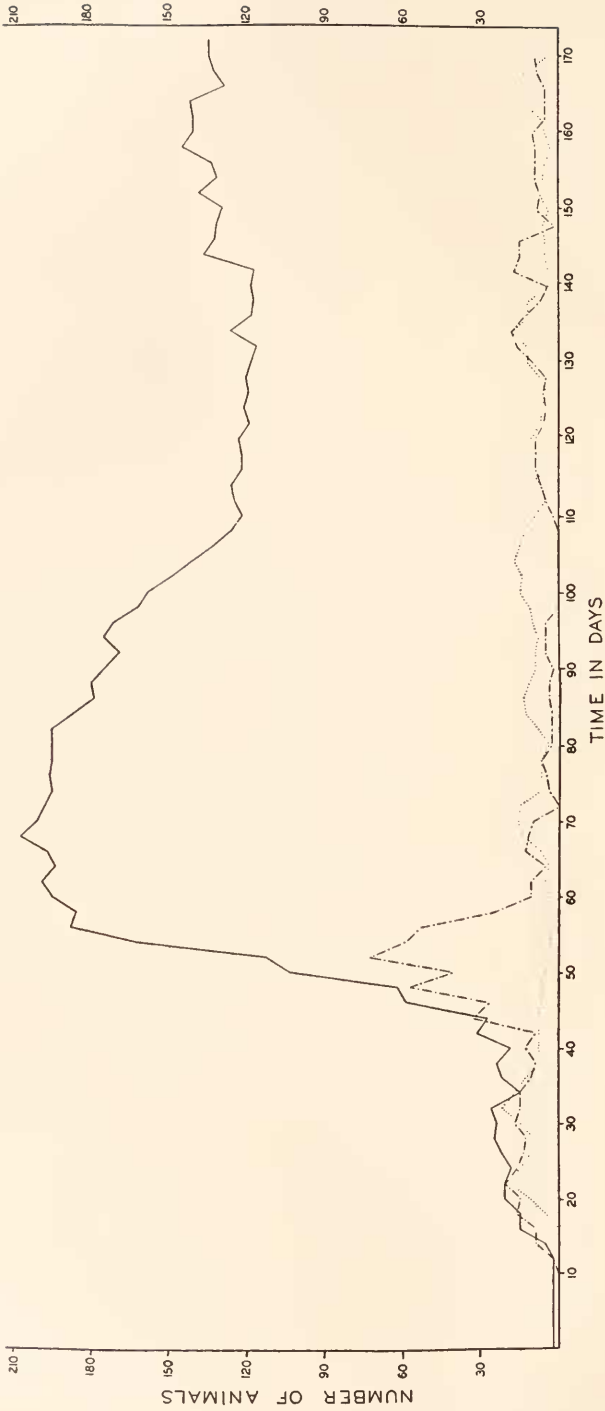
GRAPH IV.   Mechanism of oscillation at 18° C.

Legend: population size ——————
deaths *   . . . .
births *   . — . . — . . — . . — .

* Plotted as in Graph III.

NUMBER OF ANIMALS

tween them is immaterial when compared with the variations observed in the births curve. Thus the principal changes in the size of the population are attributable to fluctuations in the number of births, while the number of deaths per day remains approximately constant. This mechanism of oscillation should be contrasted with the alternating fluctuations, approximately equal in amplitude, in the numbers of daily births and deaths that constitute the mechanism of oscillation at 25°.

The lowering of the temperature to 18° exerts a damping effect upon the magnitude and persistence of oscillation that characterizes population development at 25°. At either temperature the waxing population reaches a size that it is incapable of sustaining indefinitely. Whereas at 25° this maximum was generally less than 100 and never exceeded 126, all the 18° populations attained peaks approximately twice as high, covering the range 184–241. At 25° the effects of previous crowding upon both reproduction and longevity manifest themselves quite suddenly, and a sharp peak is described. At the lower temperature crowding acts upon reproduction alone, and its full expression is delayed longer than at 25°, with the result that the population maintains its maximum long enough to describe a short "plateau." Furthermore, whereas the 25° curve sinks almost to the baseline before increase is again possible, the waning 18° population succeeds in halting its decrease at a density which it can maintain, with minor oscillations, for at least 78 days. It should be noted that this density is greater than that achieved in any of the 25° maxima. Thus regardless of temperature, the waxing population overshoots the density of potential stabilization, but the waning population at 18° does not undershoot it as the 25° population does. This virtually terminates oscillation at the lower temperature after the first peak, in sharp contrast to the continued and progressively increased oscillation at 25°.

Influence of Temperature upon Longevity and Reproduction

To account for the observed differences in the histories of populations at 18° and 25°, a series of experiments was undertaken to determine the effect of these temperatures upon the two primary variables in population growth, namely duration of life and the reproductive rate. Since such an investigation must take into consideration the influence of population density if it is to accomplish its ultimate purpose, the experiments were so designed as to measure, at each of the temperatures tested, the mean longevity and reproductive rate of animals living at different constant densities.

The desired number of parthenogenetically produced female Daphnia were placed in 50 cc. of fresh pond water with Chlorella added, not more than 12 hours after their release from the maternal brood pouch. The medium was renewed at two-day intervals, and the dead animals and young, when they appeared, were removed and counted daily. Population pressures of more than one animal per bottle were maintained constant by the introduction of substitute animals to take the place of those that had died. The problem of distinguishing these "substitutes" from the extant original members of the controlled population was surmounted by staining them with Neutral Red, a vital stain which in concentrations sufficient to dye the animals apparently did not injure them. (When fed only Chlorella, *Daphnia magna* does not develop the rich red color generally charac-

teristic of the species.)   A staining period of 12 to 24 hours in pond water tinged with a few drops of a concentrated Neutral Red solution rendered the animals distinguishable from untreated individuals for several days.   Two precautions were exercised in the selection of substitutes: 1. they were matched for size with the original members still living in the population, and 2. only individuals without eggs were chosen for this purpose.   Thus, in so far as it was possible to estimate it, the substitutes' contribution to the total density effect was proportional to their number, and all of the young produced in the population were born of charter members.

A. Experiments at 25°.

Thirty tests were made at a density of one animal per 50 cc., four at densities 5 and 10, and two at densities 25, 50 and 75.

The survival curve of the 30 single animals and the average survival curves for the five higher densities are plotted on Graph V.   To facilitate a quantitative
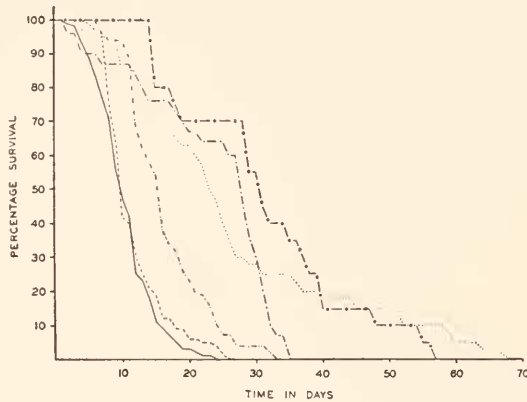
GRAPH V.   Survival at different constant population densities, 25° C.

Legend: population density   1 —·—·—·—·—·
        population density   5 —o–o–o–o–o
        population density 10 · · · · · · · · ·
        population density 25 —·· —·· —·· —
        population density 50 — — — — — —
        population density 75 —————————

comparison of survival at the six densities tested, the total number of animal days lived by each population was divided by the number of original members to give the mean duration of life at each population pressure.   These data are plotted on Graph VI, which discloses the noteworthy fact that the greatest mean longevity occurs in populations of five, rather than at the minimal density, and that animals even at a density of ten per bottle lived longer, on the average, than did those in isolation.

Two interpretations of this phenomenon suggest themselves.   MacArthur and Baillie (1929) have developed the thesis that the mean longevity of *Daphnia magna* is an inverse function of the metabolic rate and have reported (1929a) that

metabolic rate as indicated by the rate of heart beat is inversely proportional to population density in the range 1 to 25 animals per 100 cc. These authors did not determine the influence of population density upon longevity in Daphnia, but they implied that the reduced metabolic rate evinced by crowding might exert the same effect upon longevity as a metabolism lowered by some other process, such as decreasing the temperature. According to this theory, then, increased population pressure, up to the point of actual injury, might be expected to prolong life. MacArthur and Baillie's hypothesis may give the correct interpretation of the occurrence, observed in the present experiments, of the maximal longevity at a supraminimal density.

There is, however, a second possible explanation for this phenomenon. It was noted that the water in bottles containing only one animal was usually slightly clouded with bacteria, whereas the medium of larger populations was always kept

GRAPH VI.   Population density and mean longevity.
Legend: 18° C. – – – – – – –
        25° C. ————————

clear by the feeding animals. The bodies of the isolated individuals, when found dead, were frequently covered with a bacterial slime, which was occasionally observed even before death, in severe cases greatly hindering the animal's movements or even imprisoning it completely. The slime was composed of motile rods and spirilla—common fresh water saprophytes—and their gelatinous secretion. Since its appearance upon a live animal in macroscopic proportions almost invariably signalled the death of the animal within a day or two, it is believed to have contributed to the relatively high death rate at the minimal density. More crowded populations apparently never suffered from this effect; their greater numbers enabled them to maintain control of the bacterial flora.

This explanation is reminiscent of one proposed to account for a similar relation between density and mean longevity observed in populations of a different animal. Allee (1931) has suggested that the positive correlation of mean longevity with population pressure in Drosophila in the density range of 1 to 35 or 55 flies per one

ounce bottle, reported by Pearl, Miner and Parker (1927) may be due to the inability of the smaller populations to keep in check the "wild" yeasts contaminating the cultures.

The available evidence does not warrant a decision between the two interpretations, metabolic and bacterial, of the results recorded here. It is not improbable that they are both operative in the present case.

The reproductive rate of each population was calculated by dividing the total number of young produced by the number of animal days lived. Graph VII, in which the results are presented, shows that reproductive rate is an inverse function of density throughout the range in which it was determined, and it drops most rapidly as the density is increased to 25 animals per bottle.

These experiments, yielding quantitative measurements of the effects of various constant densities upon longevity and reproductive rate, offered an opportunity for studying the nature of the density effect as the limiting factor in
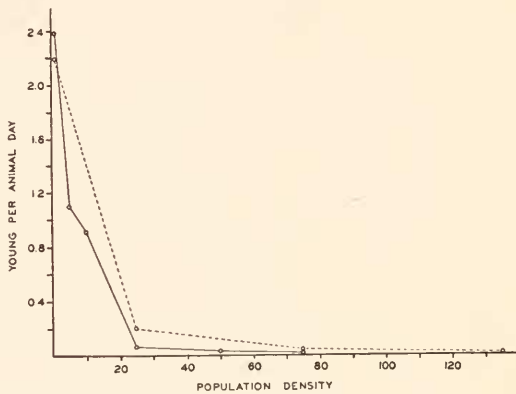
GRAPH VII.   Population density and reproductive rate.
Legend: 18° C. – – – – – – –
   25° C. ————————

population growth. In population studies with different animals a variety of density effects have been described, but almost certainly the only influence crowding can exert upon either the birth rate or the death rate of a parthenogenetic form such as Daphnia is through the conditioning of the medium. This might lead one to expect that medium which had been heavily conditioned would exert the same adverse effects upon reproduction and longevity as those brought about by actual crowding. To test this, individual Daphnia were reared in isolation in the water conditioned by the populations of different constant densities. Every other day their medium was renewed with that which one of the larger populations had been conditioning for the past two days. Contrary to expectation the isolated animals suffered thereby no impairment of reproductive capacity. Thus the conditioning of the medium is only temporary, and probably consists in the accumulation of some volatile inhibitory substance, such as carbon dioxide, or the depletion of the dissolved oxygen supply.

Further experiments have been planned to ascertain more exactly the nature

of this limiting factor.   Moreover, individual Daphnia are to be raised in medium effectively connected with that of animals living under crowded conditions, so as to test the possibility of a density effect induced directly by crowding *per se*, rather than indirectly through the conditioning of the medium.

B. *Experiments at 18°.*

Twenty-five tests were run at a density of one animal per 50 cc., two at densities 5 and 10, and one at density 135.   On the 81st day of observation, when these experiments had to be discontinued for lack of time, all the animals at the minimal density were dead, but some individuals were still living at each of the three higher densities.   The data are therefore complete for density 1 but must be regarded as partial only for densities 25, 75, and 135.   Had the experiments gone to completion, the mean longevities for the latter three densities obviously would
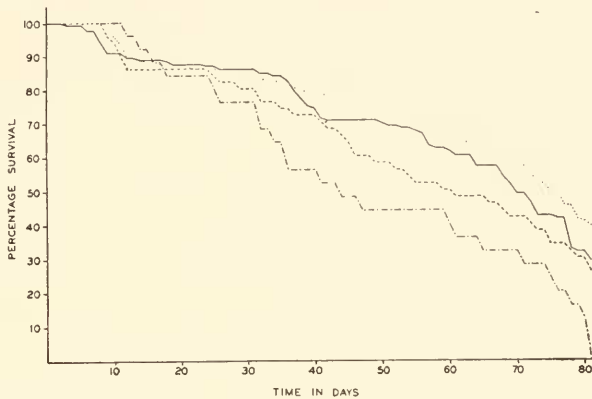
GRAPH VIII.   Survival at different constant population densities, 18° C.

Legend: population density   1 —·—·—·—·—·
                population density   25 — — — — — —
                population density   75 ··········
                population density 135 ——————

have been higher than those obtained, and it is impossible to say whether the average reproductive rates would have differed appreciably from those observed, and in which direction.   Of the two sets of data, those concerning reproduction may perhaps be considered the more accurate.

The range of densities tested was extended to include the mean equilibrium value, 135 (see Table I, p. 126), in the 18° populations.   The survival curve of the 25 single animals and the average survival curves for the other densities are plotted on Graph VIII.

Calculation of the mean longevity by dividing the total number of animal days by the density of the population yields results which, for densities 25, 75 and 135 are obviously only minimal, since some animals were still living at those densities when observations ceased.   The data are shown in Graph VI.

The mean reproductive rates for the 81 days of observation are presented in Graph VII.

C. *Comparison of results, 25° and 18°.*

Throughout the range of population densities tested, duration of life is greater at 18° than at 25° (Graph VI). This results from the positive correlation of metabolic rate with temperature and the negative correlation of longevity with metabolic rate.

The action of population pressure is quite different at the two temperatures. Whereas a density of 5 was found to be the optimum for duration of life at 25°, throughout the density range 1 to 75 longevity at 18° is a direct function of population pressure, and animals living at density 135 lived, on the average, longer than did those in isolation. The bacterial contamination of the medium which is believed to have contributed to the death rate of single individuals at 25° was never observed in the 18° bottles. This is, then, an indirect effect of temperature upon duration of life: even at the minimal density the colder water did not support a bacterial flora sufficiently concentrated to injure the animals. The absence of a bacterial effect at 18° makes it seem likely that the positive correlation of density and longevity in this case is incidental to a lowered metabolic rate. At 18° the population pressure at which metabolism is depressed to the point of positive injury lies somewhere in the density range 25 to 135, perhaps at about 75 animals per bottle.

Whereas a decrease in temperature of 7° raised the mean longevity, presumably through depression of the metabolic rate, it did not thereby bring about an equivalent reduction in reproductive rate. Apparently the rates of reproduction and mortality are not dependent upon exactly the same physiological processes. If they were, a given increase in longevity with a reduction in temperature would be correlated with a decrease in birth rate of the same magnitude.

The action of increasing density upon reproductive rate is very similar at the two temperatures (Graph VII), although it is slightly more severe at 25°: at the minimal density birth rate at 25° is higher than at 18°, but drops faster with increased crowding and at density 25 is slightly lower than the corresponding 18° rate.

The relative potential rates of population increase (i.e. the rates that would obtain if there were no density effects) can be calculated by comparing the data for the minimal density at the two temperatures. The birth rate at 18° is 2.19 young per animal day, or a gross factor of daily increase of 2.19X. The death rate (which is the reciprocal of the mean longevity, or 1/47.6) is .021X per day. Thus the net rate of potential daily increase (2.19X–.021X) is 2.17X. At 25°, gross increase (2.38X) minus death rate (.044X) yields a net rate of potential daily increase of 2.34X. When one considers that birth rate at 18° is only slightly less than at 25° and that longevity at 18° is more than twice as great as at 25°, this result is perhaps astonishing, but it illustrates the fact that birth rate is so much greater than death rate as to be the only effective factor in the net rate of increase. The reproductive rate taken alone gives the 25° population an initial advantage of .19X (2.38X–2.19X) over the 18° population. Granting the observed 25° death rate of .044X, the net rate of potential increase at 18° could not equal that at 25° even if death rate at the lower temperature were reduced to zero. But, as we have seen, the differential action of population density is such that ultimately a population attains a greater size at 18° than at 25°.

It will be recalled that oscillation at 25° was brought about by an alternation of approximately equivalent effects of population density upon the number of births and the number of deaths.   The results of the reproduction and longevity experiments at 25° bear out the contention that population density at that temperature affects both of the primary variables in population growth, in opposite directions and to approximately the same extent.   On the other hand, the reproduction and longevity experiments at 18° give results consistent with the observation that the mechanism of oscillation at that temperature was the fluctuation in the number of births about a nearly constant daily number of deaths.   Population pressure has a relatively insignificant effect upon mortality. Moreover, if the number of deaths per day in an increasing population remains constant, the death *rate* must be an inverse function of population density.   This deduction is supported by the fact that mortality in the reproduction and longevity experiments was lower at density 135 than at the minimal population pressure. This action of density upon death rate, operating in generally the same direction as the effect of density upon birth rate, tends to moderate, rather than intensify, the severity of oscillation.   Therefore oscillation at 18° must be attributed wholly to changes in birth rate.

From the reproduction-longevity data one can calculate theoretical asymptotes for populations at 18° and at 25°.   The number of young produced by an individual of mean longevity and reproductive rate at a given density can be determined by dividing the total number of young born at that density by the size of the population.   These figures for the four population pressures tested at 18° C. are as follows:

density	1	25	75	135
average number of young individual	104.5	10.7	0.73	0.19

Obviously a population of such density that each member could just replace itself before dying should be capable of maintaining a constant size.   It is found by interpolation that the density at which the average animal produces one young in the course of its life is 73.6.   It should be noted, however, that this theoretical asymptotic value is considerably lower than the mean of equilibrium values (135.4) actually established in the 18° populations.   The discrepancy is serious, and perhaps cannot be entirely explained by the fact that the reproduction-longevity experiments did not go to completion.

At the higher temperature the agreement between observed equilibrium values and the theoretical asymptote is much closer.   The number of young produced per individual in the 25° reproduction-longevity experiments is as follows:

density	1	5	10	25	50	75
average number of young individual	53.9	26.7	23.1	0.82	0.26	0.08

The calculated asymptote is 24.8 animals per bottle, while the mean of equilibrium values actually observed in the 25° populations is 29.3.

The explanation for the discrepancies between observed and calculated equilibrium values is not clear.   It is suggested that the age-structure of the population is a significant factor.   Apparently the conditions implied by a density of a given number of animals of the same age are different from those implied by a density of the same number of animals of different ages.   Although the repro-

duction-longevity experiments at both temperatures yield theoretical equilibrium values that are probably lower than the actual levels of stabilization, these experiments undoubtedly give a faithful picture of the relative effects of different densities upon the reproductive rate and upon longevity, and the data for the minimal densities can be regarded as absolute, under the given conditions.

Discussion

A. Oscillation.

Fluctuations in the density of populations in nature can usually be assigned to changes in environmental forces, which may be physico-chemical or biotic. The environmental disturbance may evoke an immediate response in the numbers of the species under consideration (as in the case of epidemics, sudden changes in meteorological conditions, etc.), or its action may be delayed for a longer interval. An example of this second category is the determination of the future size of adult populations of marine fishes by the effects of various environmental agencies upon the early developmental stages (Hjort, 1914; Johnstone, 1928). Presumably the periodic oscillations in the numbers of fur-bearing mammals and game birds which have been synchronized with sunspot cycles (Elton, 1924; Gross, 1931; Naumov, 1939; Braestrup, 1940; Green and Evans, 1940) result from complexes of environmental vectors whose action is more or less delayed.

When one considers the instability of the environment, it is not surprising that natural populations undergo violent fluctuations. However, it has been argued from mathematical grounds that the interaction of two or more animal species, e.g. predator and prey (Volterra, 1926) or parasite and host (Nicholson, 1933) is such as to give rise to rhythmic pulsations in the numbers of the animals, even though the environment is maintained constant in all other respects. In such a situation, oscillation in population density would be attributable to biotic forces exclusively, the physico-chemical factors of the environment being fixed.

Of the cases of fluctuations in numbers whose cause has been ascertained, all that have come to my attention are laid to variations in some external agency. The oscillations in Daphnia populations discussed in this paper are of an essentially different nature. Here the agent of fluctuation is internal and intrinsic. While the environment plays an important role, it is an environment whose critical changes are determined by the activities of the animals themselves. The cause of oscillation is the delay in the action of population density upon mortality and the reproductive rate, rather than a variation in some external environmental agency. It is obvious that fluctuation would not occur if the effects of a given density upon birth and death rates manifested themselves immediately; an increasing population would gradually develop an asymptote instead of "overshooting." Thus the ultimate source of oscillation is a lack of synchronization of a physiological state with the forces that provoke it.

B. Influence of temperature on population size.

It was originally intended to obtain a quantitative expression of the influence of temperature on population size by comparing the asymptotes developed at the different temperatures. Since this is clearly impossible, apparently the most satisfactory comparison would be one involving the mean sizes of the populations.

The average sizes of the 21 populations at 25° covered a range of 18.5 to 43.0, with the average at 32.6.   The range of average sizes of the 16 populations at 18° was 104.7 to 126.2, the mean 112.4.   Thus the mean of population size at 18° was about two and one half times as great as at 25°.

This result is consistent with the common experience that populations, and particularly those of marine plankton, attain greater densities in cold than in warmer regions (Oltmanns, 1923; Bělehrádek, 1935; Welch, 1935; Russell and Younge, 1936; Hesse, Allee and Schmidt, 1937).   It should be borne in mind that this greater abundance in polar waters refers to the size of the equilibrium population, rather than to the productivity in terms of the rate of turnover.

Inasmuch as the cause of this relative abundance is still not certain, and a variety of theories have been developed to account for it, the possibility of applying the results of the present study to the problem should be of considerable interest.   At the outset, however, it is apparent that the type of environmental factor preventing unlimited growth in these experimental populations (i.e. a biological conditioning of the medium by the accumulation of metabolites and/or depletion of the oxygen supply) is probably never an effective limiting factor in the open ocean.   In recent years, however, it has been contended that the latitudinal variation in plankton abundance so often observed in the sea obtains in fresh waters as well (Welch, 1935), and it is quite possible that the limiting factor in the Daphnia populations is operative in some fresh water situations.

Of the various hypotheses advanced by the oceanographers, there is but one which might be applicable to the present case.   This is the theory that attempts to explain the greater asymptotic level of polar planktonic populations by a direct effect of temperature upon the metabolic rate.   It is argued that the lower metabolism in cold waters results in a longer duration of life and thus in an accumulation of generations; and further, that this increase in longevity more than offsets the concomitant reduction in reproductive rate.   In short, the decrease in temperature exerts a greater effect on duration of life than upon the birth rate. Loeb (1912) supported this argument with the observation that the prolongation of life of sea urchin eggs with a drop in temperature greatly exceeded the retardation of their development.   The theory involves only the *direct* effects of temperature upon birth and death rates.   It alleges to explain the observed results without reference to any action of population density upon reproduction and duration of life.

A critical examination of the Loebian theory discloses that it really cannot account for differences in asymptotic levels.   The disproportionately greater longevity at the lower temperature cannot possibly influence the height of the asymptote, since birth and death rates in a population that has attained an asymptote are equal.   The equilibrium level is determined by two factors: the previous rate of increase of the population and the duration of that increase.   The birth rate/death rate ratio determines the rate of population increase.   It is in this ratio that the disproportionately great longevity at the lower temperature would express itself, yielding a greater net rate of population increase in colder than in warmer waters.   But the second factor, namely the duration of population increase, is in no way affected by the birth rate/death rate ratio.   It is determined by some limiting factor in population growth other than temperature.   This limiting factor may, in turn, be influenced by temperature, but it is essentially a

result of population density. Without it, that is with no limit to the duration
of increase, the population would continue growing, geometrically and indefinitely.
Since Loeb's theory involves only the rate of increase and disregards the factor of
duration of increase it makes no provision for any check in population growth.
The inescapable conclusion is an everlasting logarithmic increase. From a
slightly different point of attack this criticism may be rephrased thus. Since
there are two factors involved in the asymptotic level ultimately attained, a
population with an infinitesimally low rate of increase may eventually reach a
greater asymptote than that developed in shorter time by another population with
a much higher rate of increase. As an example, in the present experiments the
potential rate of increase at 25° was found to exceed that at 18°, yet populations
at the latter temperature attained the greater mean size. It should be pointed
out, however, that owing to seasonal phenomena, the time element may be
critical in the development of some populations in nature. Because of the
brevity of the favorable season, these populations may never reach their potential
asymptotes. In this case the rate of increase is the all-important factor in the
size of the population at any given moment.

The explanation for the greater mean size of the 18° populations would appear
to be a differential action of density at the two temperatures. The reproduction-
longevity experiments revealed that increasing population pressure exerts a more
severe effect upon the birth and death rates at 25° than at 18°. Possibly this
result is related to the difference in solubility of atmospheric oxygen in the medium
at different temperatures, but whatever the nature of the conditioning may be, the
influence of temperature upon mean population size is indirect. It operates
through the differential effects of population pressure. Thus the difference in
temperature exerts its observed influence upon the mean of population size only
by modifying the action of population density.

Summary

1. The development of populations of *Daphnia magna* was followed at two
different constant temperatures. Sixteen populations were maintained at 18° and
21 at 25° C. The 50 cc. of pond water which served as medium were renewed
every other day and always contained an excess quantity of the food-alga Chlorella.

2. Population development at 25° proved oscillatory in nature, four peaks
occurring in 234 days, with a maximum population size of 126 animals. In the
174 days of observation at 18°, one major peak was observed (maximum 241)
followed by a decrease and virtual stabilization at a population density of
about 135.

3. Analysis of the oscillation disclosed that it is due to a delay in the expression
of the effects of population density upon birth and death rates.

4. The mechanism of oscillation at 25° is an alternation of fluctuations in
numbers of births and numbers of deaths. The mechanism at 18° is the fluctua-
tion in the number of births about a nearly constant number of deaths.

5. Experiments with a series of population densities artificially maintained
constant showed that birth rate at 25° is an inverse function of population density.
At 18° the effect of density is similar but less severe.

6. Under these conditions of constant density, mortality at 25° is in general a
function of population density, although the minimal mortality occurs at a

density of 5. At 18° mortality is but little affected by conditions of density, and is apparently least at about 75 animals/50 cc.

7. The mean of population size at 18° was two and one half times as great as that at 25°.

8. This fact is compared to the supposed greater density of planktonic populations in polar than in tropical waters. The results of this study cannot be applied to the problem of marine plankton abundance since the limiting factor in the present case (the conditioning of the medium by the accumulation of metabolites and/or depletion of the dissolved oxygen supply) is presumably never operative in the ocean, although it may be operative in some fresh water situations.

9. The possibility of accounting for the greater mean size of the 18° populations by reference to the direct effect of temperature upon longevity is considered but rejected. A basic fallacy is pointed out in the theory which attempts to explain by such a direct effect of temperature the greater density of asymptotic populations in polar than in tropical regions.

10. It is concluded that the influence of temperature upon mean population size observed in these experiments is indirect: the temperature difference exerts its effect only by modifying the action of population density.

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